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THE USE OF VITAL STAINING OF TISSUE CULTURE WHEN WORKING WITH THE MEASLES VIRUS

[Following is the translation of an article by V. B. Seybil and V. I. Khozinskiy, Institute of Poliomyelitis and Viral Encephalitises, AMN, USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), No 2, 1967, pages 246-249. It was submitted on 14 Mar 1966.]

When working with the measles virus the investigator is faced with the difficult task of calculating the cytopathic changes in the tissue culture. With a small inoculating dose of virus the cytopathic action is limited to the formation of only individual symplasts in the tissue culture. By the end of the period of observation the number and size of these are increased insignificantly. The difficulties during microscopic investigation are connected with the small degree of contrast of the unstained tissue culture (Fig. 1).

We developed a method for the vital staining of tissue culture. Here against a background of intensively stained protoplasm the unstained nuclei and contours of individual cells stand out clearly (Fig. 2).

The developing symplasts are detected easily against the background of the normal culture in the form of sectors of intensively stained protoplasm with the accumulation of a greater or lesser number of nuclei. Here the absence of intracellular boundaries is clearly evident. (Fig. 3) The proposed method considerably eases and speeds up the microscopic investigation of inoculated tissue culture. It can be used both during titration and identification of viruses and during titration of antibodies in serum with a calculation of results based on the cytopathic effect.

For the work we used the primary tissue culture of monkey kidneys (KTPO), human diploid cells (DKCh), and transplanted cell cultures: kidneys from a green marmoset (POZM), HeLa, and others. Tissue cultures were cultivated in test tubes using the nutrient growth medium usually used for each of these cultures. After inoculation of the KTPO the supporting medium consisted of an Erla solution with an 0.5% solution of milk albumin hydrolyzate, 5% normal bovine serum, and a 1.5% solution of neutral red in a dilution of 1:1,000. For economy of the medium with milk albumin hydrolyzate we recommend a mixture of the following composition: Erla solution in a 10-fold concentration - 10%, distilled water - 83.4%, normal bovine serum - 2%, an 0.1% solution of neutral red - 1.5%, and a solution of sodium bicarbonate - 3.1%. This mixture makes it possible

to maintain the cells in good condition for 7-10 days. In those cases when cultures of transplated HeLa, DKCh, or POZM cells were used the supporting medium consisted of Igla medium (63.6-88.6%), a medium with a 0.5% solution of milk albumin hydrolyzate in an Erla solution (30-5%), normal bovine serum (5%), and an 0.1% solution of neutral red (1.4%).

Fig. 1. Symplasts of measles virus in an unstained tissue culture of monkey kidney.

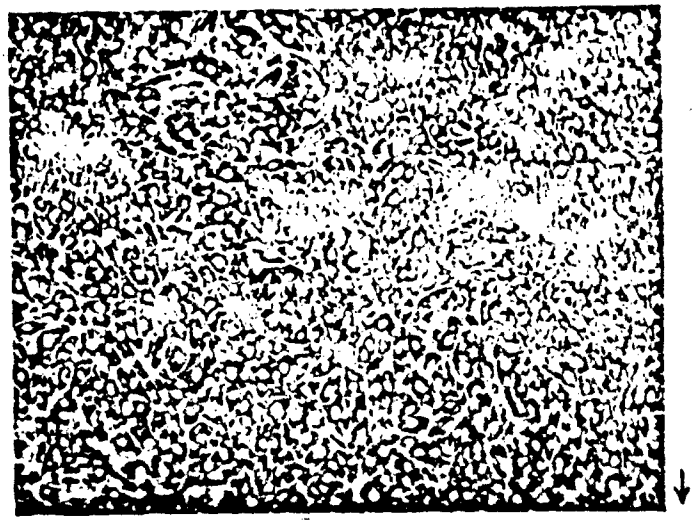

Fig. 2. Normal culture of monkey kidney stained vitally with neutral red.

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Fig. 3. Symplasts of measles virus in a stained tissue culture of monkey kidney.

After inoculation the test tubes were incubated in a non-illuminated incubator. During the course of the experiment the tissue culture was examined microscopically twice. During the first examination the degree of illumination for the microscope field of vision was reduced somewhat. It is known that the measles virus possesses a specific degree of light sensitivity, however, the photodynamic effect of neutral red under these conditions did not influence the multiplication of vaccine and "wild" strains of the measles virus. During a parallel titration of viruses in test tubes with the supporting medium containing neutral red and the usual medium a reliable difference in titer was absent. The titer if virus, established in the stained tissue culture, usually was somewhat higher, which was probably the result of the more complete exposure of symplasts (see table).

The proposed method does not require an additional expenditure of labor and time for staining of the preparations and makes it possible to follow the dynamics of cytopathic changes in the stained tissue culture.

Results from the titration of viruses when useing two supporting mediums. (a)

(a) Результаты титрования вирусов при использовании двух поддерживающих сред

(b) Штамм вируса кори	(c) Титр вируса (в lgТЦИД$_{50}$/мл)	
	(d) обычно применяемая среда	среда с добавлением нейтрального красного (e)
(f) Эдмонстон В	3,66	3,83
(g) Эдмонстон НА-9	3,66	3,66
(h) Шварц	4,00	4,50
(i) Ленинград-16	3,50	3,50
(j) Прага-2	4,50	4,50
(k) Москва-6	3,66	3,66
(l) Рэб	3,83	4,33

Key: (b) Strain of measles virus; (c) Titer of virus (in lgTCIF$_{50}$/ml) [the acronym TCID is not confirmed]; (d) medium normally used; (e) medium with the addition of neutral red; (f) Edmonston B; (g) Edmonston HA-9; (h) Schwarz; (i) Leningrad-16; (j) Prague-2; (k) Moscow-6; (l) Ryab.